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(54) **METHOD, DEVICE AND SYSTEM FOR VOLUMETRIC ENUMERATION OF WHITE BLOOD CELLS**

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See application file for complete search history.

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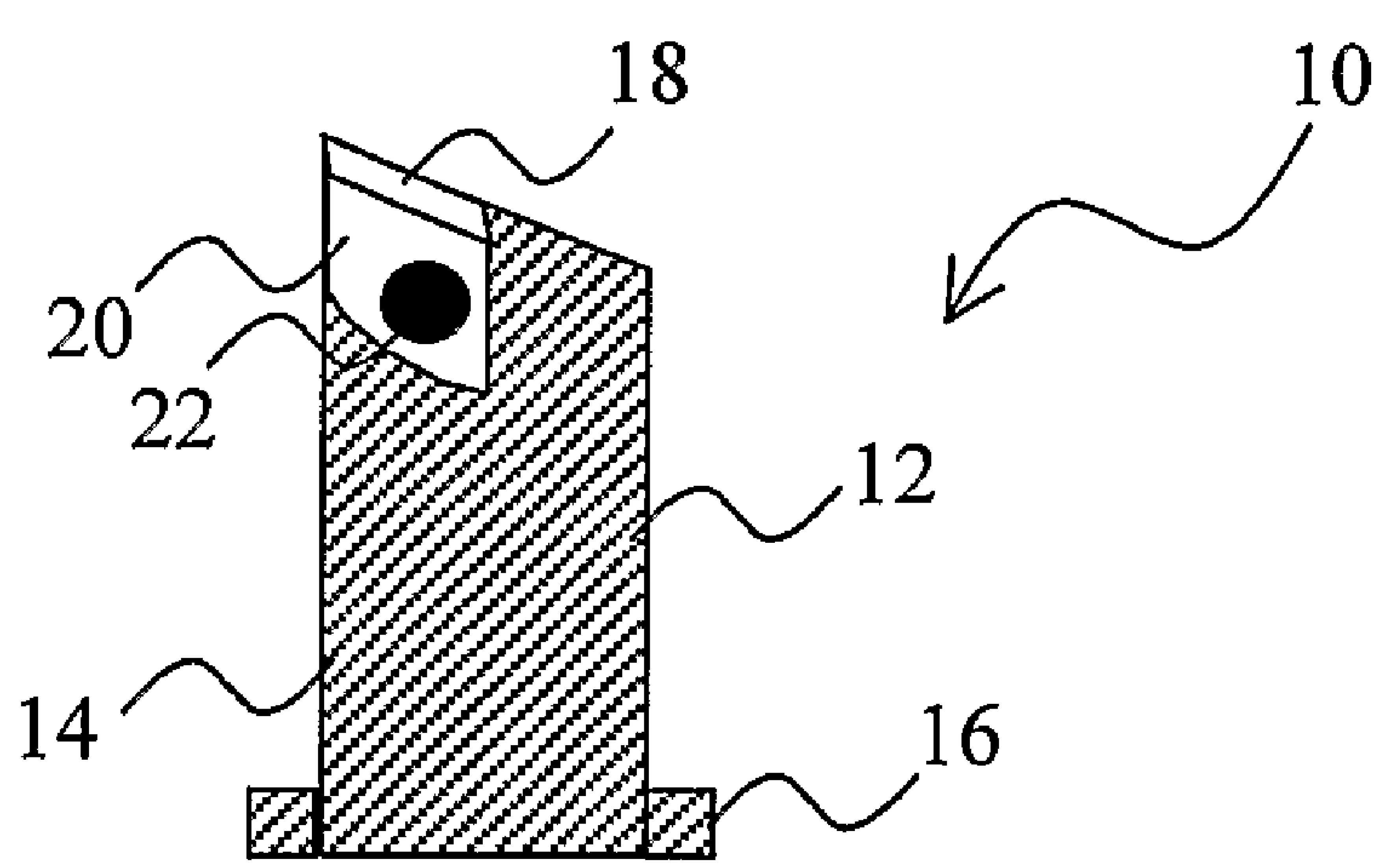
(57) **ABSTRACT**

A sample acquiring device for volumetric enumeration of white blood cells in a blood sample comprises a measurement cavity for receiving a blood sample. The measurement cavity has a predetermined fixed thickness. The sample acquiring device further comprises a reagent, which is arranged in a dried form on a surface defining the measurement cavity. The reagent comprises a hemolysing agent for lysing red blood cells in the blood sample, and a staining agent for selectively staining white blood cells in the blood sample. A system comprises the sample acquiring device and a measurement apparatus. The measurement apparatus comprises a sample acquiring device holder, a light source, and an imaging system for acquiring a digital image of a magnification of the sample. The measurement apparatus further comprises an image analyser arranged to analyse the acquired digital image for determining the number of white blood cells in the blood sample.

23 Claims, 3 Drawing Sheets



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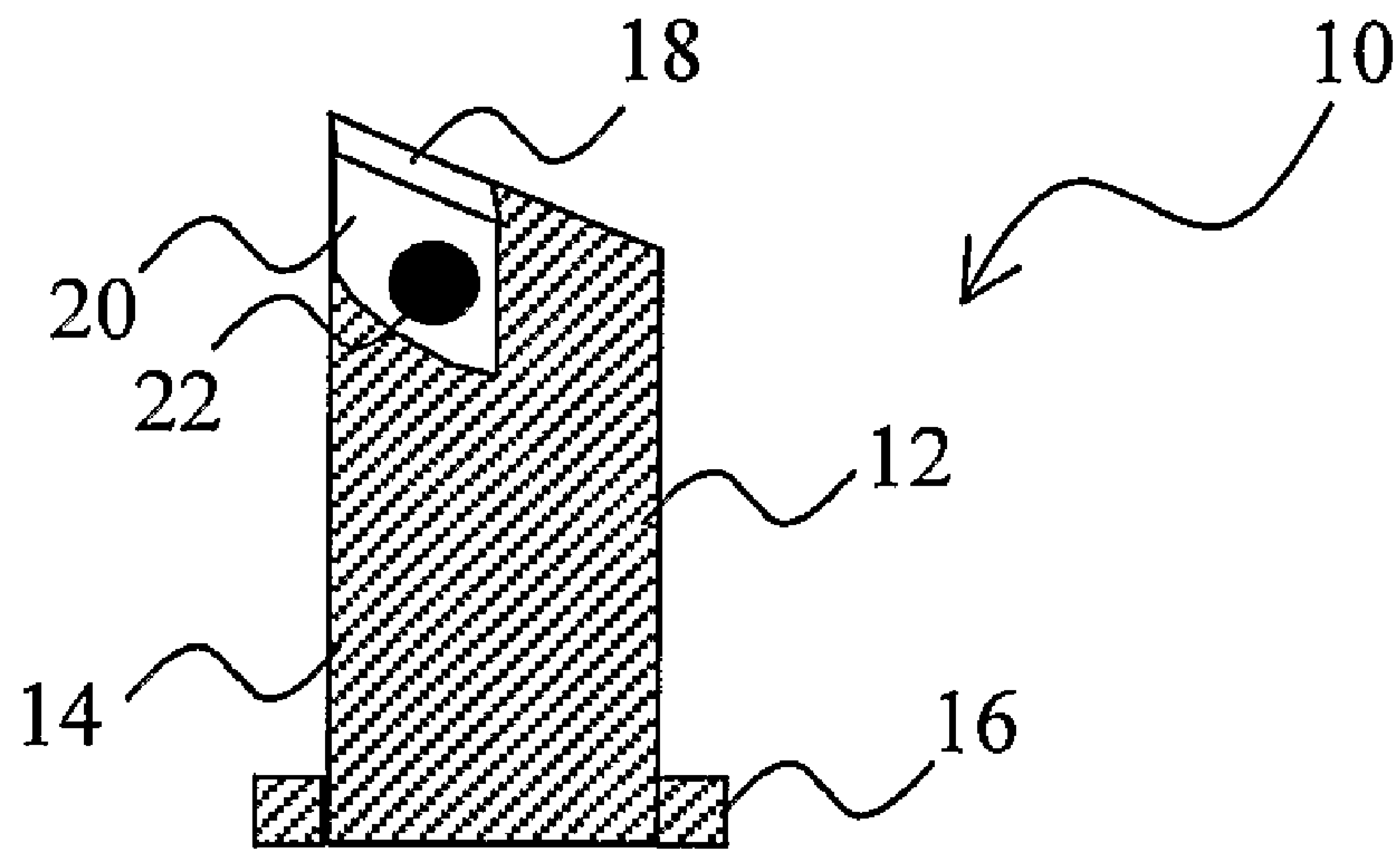

Fig. 1

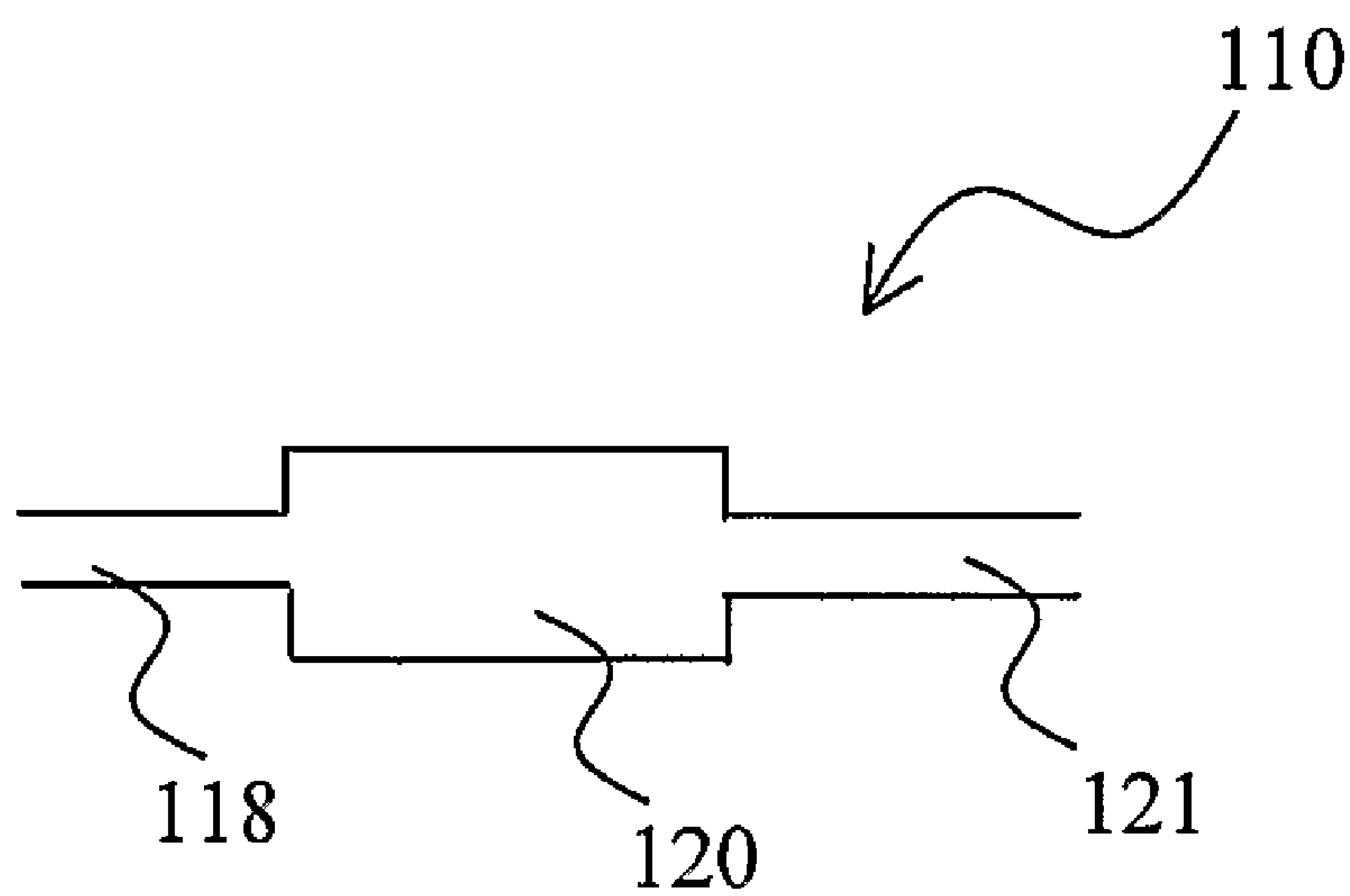

Fig. 2

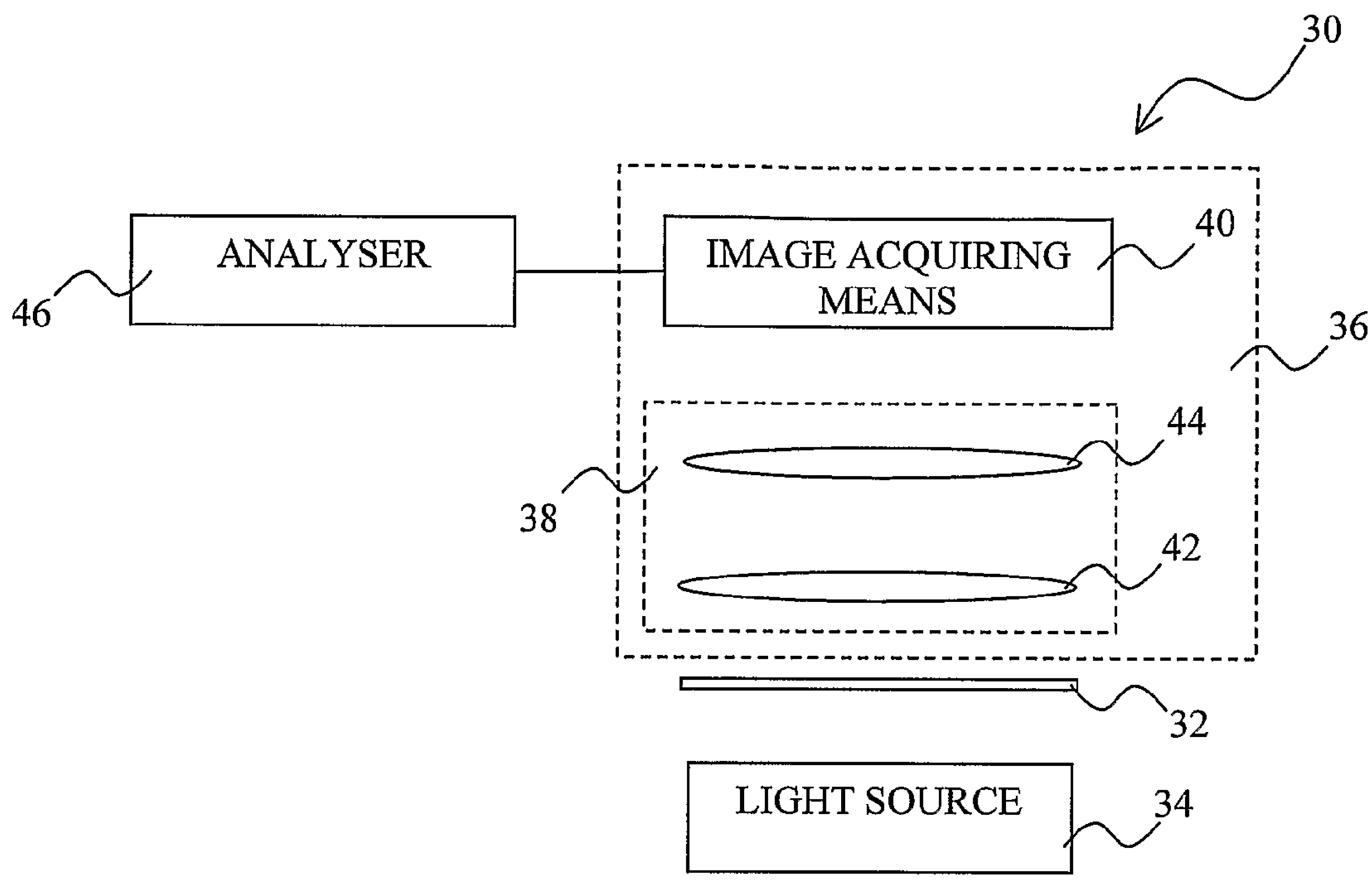
30
ANALYSER
46
IMAGE ACQUIRING MEANS
40
36
44
38
42
32
LIGHT SOURCE
34

Fig. 3

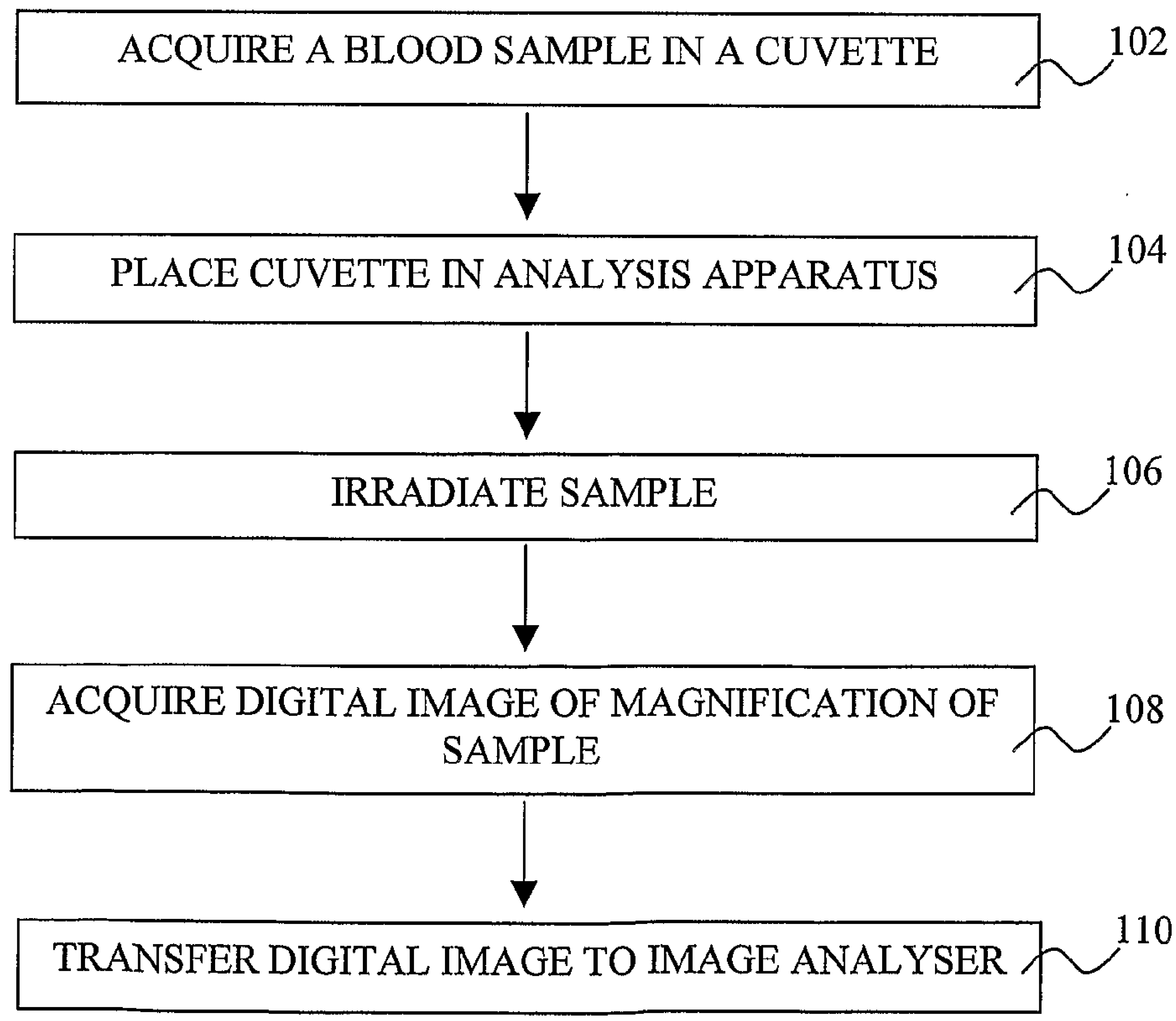
ACQUIRE A BLOOD SAMPLE IN A CUVETTE
102
PLACE CUVETTE IN ANALYSIS APPARATUS
104
IRRADIATE SAMPLE
106
ACQUIRE DIGITAL IMAGE OF MAGNIFICATION OF SAMPLE
108
TRANSFER DIGITAL IMAGE TO IMAGE ANALYSER
110

Fig. 4

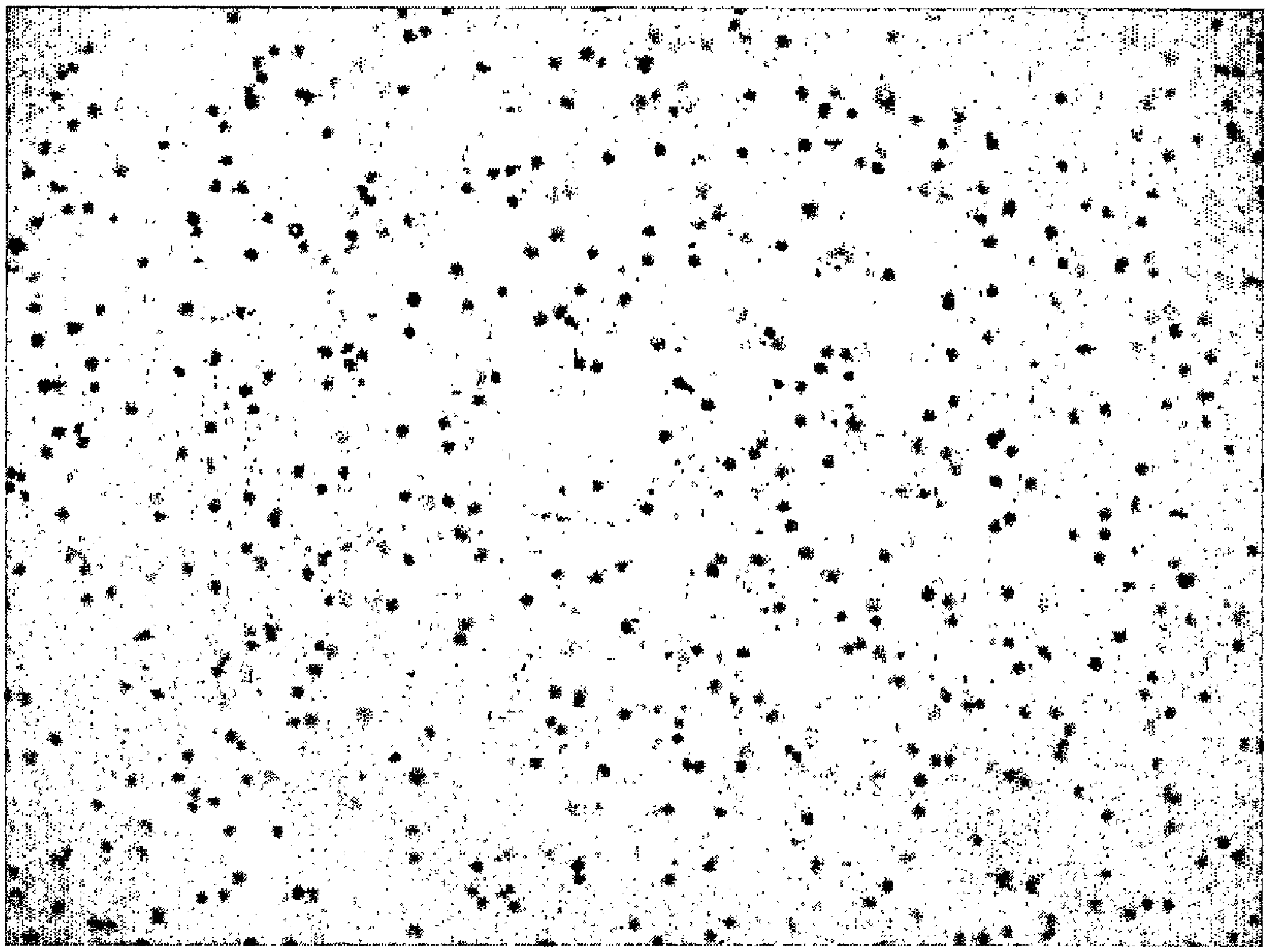

Fig. 5

METHOD, DEVICE AND SYSTEM FOR VOLUMETRIC ENUMERATION OF WHITE BLOOD CELLS

TECHNICAL FIELD

The present invention relates to a sample acquiring device, a method and a system for volumetric enumeration of white blood cells in a blood sample.

BACKGROUND OF THE INVENTION

Determining a white blood cell count is often important in connection to treating a patient. This analysis may be needed for diagnosing e.g. leukaemia, or infectious or inflammatory diseases or for monitoring treatments. It is desirable to enable analysis results to be obtained as quickly as possible in order to minimize waiting times for patients and enabling a physician to make a decision of treatment and diagnosis directly when making a first examination of the patient. It would therefore be preferable to provide an analysis method which may be quickly performed by the physician or a nurse without the need of sending a test away to a laboratory.

Today, a white blood cell count is normally obtained through a manual procedure by staining a blood sample and microscopically viewing the sample in a special counting chamber, e.g. a Bürker chamber. The counting chamber is provided with a grid dividing the chamber in well-defined small volumes. The white blood cells are allowed to settle at the bottom of the counting chamber in order to enable the microscope to focus on all cells in the chamber and, thus, facilitate counting. Thus, the sample need to settle for several minutes before performing the counting. The white blood cell count can then be determined by counting the number of blood cells per box in the grid. The white blood cell count is obtained manually by an analyst, who needs to be experienced in performing the analysis in order to be able to perform a reliable analysis.

This analysis is time-consuming. Further, since it is performed manually, the results of the analysis may vary depending on the person performing the analysis.

There are a few number of existing automated analysis methods for determining a white blood cell count. The white blood cell count may be determined by means of the Coulter principle, which is based on determining cell size and thereby the cell type by sensing an impedance. A method for counting white blood cells by the Coulter principle is described in U.S. Pat. No. 5,262,302.

The Coulter principle is the dominating, automated analysis method that is presently being used. However, there are a few other methods that have been described. One such method for determining a white blood cell count is disclosed in U.S. Pat. No. 5,585,246. Here, a blood sample has to be prepared by being mixed with a fluorescent dye and ligand complex which tags the white blood cells. The sample is introduced into a capillary and is irradiated by a laser source which scans over the sample in the capillary. The fluorescence is measured in order to determine the number of white blood cells. A similar method is disclosed in WO 97/02482, using a fluorescent dye and a laser source scanning over a capillary. This method is adapted for enumeration of white blood cells in apheresis products containing a low number of white blood cells. Here, the capillary is quite thick and it is necessary to wait until the white blood cells have settled at the bottom of the capillary before the capillary may be scanned.

In WO 99/45384, a sample containing chamber having varying thickness is shown. The varying thickness separates different compounds of blood. The blood sample is stained with a colorant to differentially highlight at least three different white blood cell types in the blood sample. The white blood cells may be enumerated by using an optical scanning instrument to view a portion of the chamber.

There is still a need to speed up and simplify existing automated methods for determining a white blood cell count such that analysis may be provided at point of care. Further, since the white blood cell count is such a commonly performed analysis, any improvement to the analysis method would have a great impact on patient care. An analysis method providing a possibility to obtain results at point of care would be particularly advantageous.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a simple analysis for determining a volumetric enumeration of white blood cells. It is a further object of the invention to provide a quick analysis without the need for complicated apparatuses or extensive sample preparations.

These objects are partly or wholly achieved by a sample acquiring device, a method and a system according to the independent claims. Preferred embodiments are evident from the dependent claims.

Thus, there is provided a sample acquiring device for volumetric enumeration of white blood cells in a blood sample. The sample acquiring device comprises a measurement cavity for receiving a blood sample. The measurement cavity has a predetermined fixed thickness. The sample acquiring device further comprises a reagent, which is arranged in a dried form on a surface defining the measurement cavity, said reagent comprising a hemolysing agent for lysing red blood cells in the blood sample, and a staining agent for selectively staining white blood cells in the blood sample.

The sample acquiring device provides a possibility to directly obtain a sample of whole blood into the measurement cavity and provide it for analysis. There is no need for sample preparation. In fact, the blood sample may be sucked into the measurement cavity directly from a pricked finger of a patient. Providing the sample acquiring device with a reagent enables a reaction within the sample acquiring device which makes the sample ready for analysis. The reaction is initiated when the blood sample comes into contact with the reagent. Thus, there is no need for manually preparing the sample, which makes the analysis especially suitable to be performed directly in an examination room while the patient is waiting.

Since the reagent is provided in a dried form, the sample acquiring device may be transported and stored for a long time without affecting the usability of the sample acquiring device. Thus, the sample acquiring device with the reagent may be manufactured and prepared long before making the analysis of a blood sample.

Whereas many existing methods are able to count different blood cells and even subgroups of blood cells, the sample acquiring device according to the invention is specifically adapted to performing volumetric enumeration of white blood cells. The reagent comprises a hemolysing agent which will lyse the red blood cells in the blood sample. This destroys the possibilities to enumerate the red blood cells in the sample. On the other hand, the lysing of the red blood cells simplifies the distinguishing and identification of the white blood cells within the blood sample.

The staining agent provides a marking of the individual white blood cells. This enables the white blood cells to be individually viewed or detected. The white blood cells may e.g. be detected by scanning the measurement cavity or obtaining an image of the measurement cavity. The white blood cell count may thus be obtained by summing the number of individually detected white blood cells in a defined volume.

The invention also provides a method for volumetric enumeration of white blood cells in a blood sample. The method comprises acquiring a blood sample into a measurement cavity of a sample acquiring device, said measurement cavity holding a reagent comprising a hemolysing agent and a staining agent to react with the sample such that the white blood cells are stained, irradiating the sample with the stained white blood cells, acquiring a digital image of a magnification of the irradiated sample in the measurement cavity, wherein white blood cells are distinguished by selective staining of the staining agent, and digitally analysing the digital image for identifying white blood cells and determining the number of white blood cells in the sample.

The invention further provides a system for volumetric enumeration of white blood cells in a blood sample. The system comprises a sample acquiring device as described above. The system further comprises a measurement apparatus comprising a sample acquiring device holder arranged to receive the sample acquiring device which holds a blood sample in the measurement cavity, and a light source arranged to irradiate the blood sample. The measurement apparatus further comprises an imaging system, comprising a magnifying system and a digital image acquiring means for acquiring a digital image of a magnification of the irradiated sample in the measurement cavity, wherein white blood cells are distinguished in the digital image by selective staining of the staining agent. The measurement apparatus also comprises an image analyser arranged to analyse the acquired digital image for identifying white blood cells and determining the number of white blood cells in the blood sample.

The method and system of the invention provide a very simple analysis of a blood sample for determining a white blood cell count. The analysis does not require complicated measurement apparatus or advanced steps to be performed by an operator. Therefore, it may be performed in direct connection to examination of a patient, without the need for a qualified technician. The measurement apparatus utilizes the properties of the sample acquiring device for making an analysis on a sample of undiluted whole blood that has been directly acquired into the measurement cavity. The measurement apparatus is arranged to image a volume of the sample for making a volumetric enumeration of the white blood cells from the one image.

The blood sample is allowed to be mixed with the reagent in the measurement cavity. Within a few minutes or less, the reaction of the blood sample with the reagent will have hemolysed the red blood cells and stained the white blood cells such that the sample is ready for being presented to the optical measurement. The blood sample may be mixed with the reagent by e.g. dispersion or diffusion of the reagent into the blood sample or by actively vibrating or moving the sample acquiring device so that an agitation is caused in the measurement cavity.

The sample acquiring device may comprise a body member having two planar surfaces to define said measurement cavity. The planar surfaces may be arranged at a predetermined distance from one another to determine a sample thickness for an optical measurement. This implies that the sample acquiring device provides a well-defined thickness to the optical measurement, which may be used for accurately determining the white blood cell count per volumetric unit of the blood sample. A volume of an analysed sample will be well-defined by the thickness of the measurement cavity and an area of the sample being imaged. Thus, the well-defined volume could be used for associating the number of white blood cells to the volume of the blood sample such that the volumetric white blood cell count is determined.

The measurement cavity preferably has a uniform thickness of 50-170 micrometers. A thickness of at least 50 micrometers implies that the measurement cavity does not force the blood sample to be smeared into a monolayer allowing a larger volume of blood to be analysed over a small cross-sectional area. Thus, a sufficiently large volume of the blood sample in order to give reliable values of the white blood cell count may be analysed using a relatively small image of the blood sample. The thickness is more preferably at least 100 micrometers, which allows an even smaller cross-sectional area to be analysed or a larger sample volume to be analysed. Further, the thickness of at least 50 micrometers and more preferably 100 micrometers also simplifies manufacture of the measurement cavity having a well-defined thickness between two planar surfaces.

For most samples arranged in a cavity having a thickness of no more than 170 micrometers, the white blood cell count is so low that there will be only minor deviations due to white blood cells being arranged overlapping each other. However, the effect of such deviations will be related to the white blood cell count and may thus, at least to some extent, be handled by means of statistically correcting results at least for large values of the white blood cell count. This statistical correction may be based on calibrations of the measurement apparatus. The deviations will be even less for a measurement cavity having a thickness of no more than 150 micrometers, whereby a simpler calibration may be used. This thickness may even not require any calibration for overlapping blood cells.

Further, the thickness of the measurement cavity is sufficiently small to enable the measurement apparatus to obtain a digital image such that the entire depth of the measurement cavity may be analysed simultaneously. Since a magnifying system is to be used in the measurement apparatus, it is not simple to obtain a large depth of field. Therefore, the thickness of the measurement cavity would preferably not exceed 150 micrometers in order for the entire thickness to be simultaneously analysed in a digital image. The depth of field may be arranged to handle a thickness of the measurement cavity of 170 micrometers.

The digital image may be acquired with a depth of field at least corresponding to the thickness of the measurement cavity. This implies that a sufficient focus is obtained of the entire sample thickness such that the entire thickness of the measurement cavity may be simultaneously analysed in the digital image of the sample. Thus, there is no need to await that the white blood cells settle in the measurement cavity, whereby the time for making an analysis is reduced. By choosing not to focus very sharply on a specific part of the sample, a sufficient focus is obtained of the entire sample thickness to allow identifying the number of white blood cells in the sample. This implies that a white blood cell may be somewhat blurred and still be considered to be in focus of the depth of field.

The sample acquiring device may be provided with a reagent that has been applied to the surface solved in a volatile liquid which has evaporated to leave the reagent in a dried form.

It has been realised that the reagent is advantageously solved in a volatile liquid before being inserted into the measurement cavity. This implies that the liquid may in an effective manner be evaporated from the narrow space of the measurement cavity during manufacture and preparation of the sample acquiring device.

The reagent may preferably be solved in an organic solvent and more preferably be solved in methanol. Such solvents are volatile and may appropriately be used for drying the reagent onto a surface of the measurement cavity.

The staining agent may be arranged to selectively stain the nucleus of the white blood cells. This implies that the white blood cells may be identified as coloured dots and therefore easily be counted in a digital image.

The staining agent may be any one in the group of Hematoxylin, Methylene blue, Methylene green, Methylene azure, cresyl violet acetate, Toluidine blue, Gentian violet, Sudan analogues, Gallocyanine, and Fuchsin analogues, or any combination thereof. However, it should be appreciated that the staining agent is not limited to this group, but many other substances may be contemplated.

The hemolysing agent may be a quaternary ammonium salt, a saponin, a bile acid, such as deoxycholic acid, a digitoxin, a snake venom, a glucopyranoside or a non-ionic detergent of type Triton. However, it should be appreciated that the hemolysing agent is not limited to this group, but many other substances may be contemplated.

The sample acquiring device may further comprise a sample inlet communicating the measurement cavity with the exterior of the sample acquiring device, said inlet being arranged to acquire a blood sample. The sample inlet may be arranged to draw up a blood sample by a capillary force and the measurement cavity may further draw blood from the inlet into the cavity. As a result, the blood sample may easily be acquired into the measurement cavity by simply moving the sample inlet into contact with blood. Then, the capillary forces of the sample inlet and the measurement cavity will draw up a well-defined amount of blood into the measurement cavity. Alternatively, the blood sample may be sucked or drawn into the measurement cavity by means of applying an external pumping force to the sample acquiring device. According to another alternative, the blood sample may be acquired into a pipette and then be introduced into the measurement cavity by means of the pipette.

The sample acquiring device may be disposable, i.e. it is arranged to be used once only. The sample acquiring device provides a kit for performing a white blood cell count, since the sample acquiring device is able to receive a blood sample and holds all reagents needed in order to present the sample to cell counting. This is particularly enabled since the sample acquiring device is adapted for use once only and may be formed without consideration of possibilities to clean the sample acquiring device and re-apply a reagent. Also, the sample acquiring device may be moulded in plastic material and thereby be manufactured at a low price rate. Thus, it may still be cost-effective to use a disposable sample acquiring device.

The sample may be irradiated by light of a wavelength corresponding to a peak in absorbance of the staining agent. Consequently, the stained white blood cells which contain an accumulation of staining agent will be detected by a low transmittance of light.

The irradiating may be performed by means of a laser source. The laser source may provide light of a well-defined wavelength fitting the absorbance of the staining agent. Further, the laser source provides collimated light, minimizing disturbances of stray light, such that a point of low transmittance of light will be sharply distinguished.

The irradiating may alternatively be performed by means of a light emitting diode. This light source may still provide sufficient irradiating conditions for properly distinguishing white blood cells from other matter in the sample.

The digital image may be acquired using a magnification power of 3-200×, more preferably 3-10×. Within these ranges of magnification power, the white blood cells are sufficiently magnified in order to be detected, while the depth of field may be arranged to cover the sample thickness. A low magnification power implies that a large depth of field may be obtained. However, if a low magnification power is used, the white blood cells may be hard to detect. A lower magnification power may be used by increasing the number of pixels in the acquired image, that is by improving the resolution of the digital image. In this way, it has been possible to use a magnification power of 3-4×, while still enabling the white blood cells to be detected.

The analysing comprises identifying areas of high light absorbance in the digital image. The analysing may further comprise identifying black or dark dots in the digital image. Since the staining agents may be accumulated in the nucleus of the white blood cells, the absorbance of the light may have peaks at separate points. These points will form black dots in the digital image.

The analysing may further comprise electronically magnifying the acquired digital image. While the sample is being magnified for acquiring a magnified digital image of the sample, the acquired digital image itself may be electronically magnified for simplifying distinguishing between objects that are imaged very closely to each other in the acquired digital image.

BRIEF DESCRIPTION OF DRAWINGS

The invention will now be described in further detail by way of example under reference to the accompanying drawings.

FIG. **1** is a schematic view of a sample acquiring device according to an embodiment of the invention.

FIG. **2** is a schematic view of a sample acquiring device according to another embodiment of the invention.

FIG. **3** is a schematic view of a measurement apparatus according to an embodiment of the invention.

FIG. **4** is a flow chart of a method according to an embodiment of the invention.

FIG. **5** is a digital image of a blood sample to be used for volumetric enumeration of white blood cells.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Referring now to FIG. **1**, a sample acquiring device **10** according to an embodiment of the invention will be described. The sample acquiring device **10** is disposable and is to be thrown away after having been used for analysis. This implies that the sample acquiring device **10** does not require complicated handling. The sample acquiring device **10** is preferably formed in a plastic material and may be manufactured by injection-moulding. This makes manufacture of the sample acquiring device **10** simple and cheap, whereby the costs of the sample acquiring device **10** may be kept down.

The sample acquiring device **10** comprises a body member **12**, which has a base **14**, which may be touched by an operator without causing any interference in analysis results. The base **14** may also have projections **16** that may fit a holder in an analysis apparatus. The projections **16** may be arranged such that the sample acquiring device **10** will be correctly positioned in the analysis apparatus.

The sample acquiring device **10** further comprises a sample inlet **18**. The sample inlet **18** is defined between opposite walls within the sample acquiring device **10**, the walls being arranged so close to each other that a capillary force may be created in the sample inlet **18**. The sample inlet **18** communicates with the exterior of the sample acquiring device **10** for allowing blood to be drawn into the sample acquiring device **10**. The sample acquiring device **10** further comprises a chamber for counting white blood cells in the form of a measurement cavity **20** arranged between opposite walls inside the sample acquiring device **10**. The measurement cavity **20** is arranged in communication with the sample inlet **18**. The walls defining the measurement cavity **20** are arranged closer together than the walls of the sample inlet **18**, such that a capillary force may draw blood from the sample inlet **18** into the measurement cavity **20**.

The walls of the measurement cavity **20** are arranged at a distance from each other of 50-170 micrometers. The measurement cavity **20** is more preferably at least 100 micrometers thick. Further, the measurement cavity **20** is more preferably no more than 150 micrometers thick. The distance is uniform over the entire measurement cavity **20**. The thickness of the measurement cavity **20** defines the volume of blood being examined. Since the analysis result is to be compared to the volume of the blood sample being examined, the thickness of the measurement cavity **20** needs to be very accurate, i.e. only very small variations in the thickness are allowed within the measurement cavity **20** and between measurement cavities **20** of different sample acquiring devices **10**. The thickness allows a relatively large sample volume to be analysed in a small area of the cavity. The thickness theoretically allows white blood cells to be arranged on top of each other within the measurement cavity **20**. However, the amount of white blood cells within blood is so low that the probability for this to occur is very low.

The sample acquiring device **10** is typically adapted for measuring white blood cell counts above 0.5×10^9 cells/litre blood. At lower white blood cell counts, the sample volume will be too small to allow statistically significant amounts of white blood cells to be counted. Further, when the white blood cell count exceeds 12×10^9 cells/litre blood, the effect of blood cells being arranged overlapping each other will start to be significant in the measured white blood cell count. At this white blood cell count, the white blood cells will cover approximately 8% of the cross-section of the sample being irradiated, if the thickness of the measurement cavity is 140 micrometers. Thus, in order to obtain correct white blood cell counts, this effect will need to be accounted for. Therefore, a statistical correction of values of the white blood cell count above 12×10^9 cells/litre blood may be used. This statistical correction will be increasing for increasing white-blood cell counts, since the effect of overlapping blood cells will be larger for larger white blood cell counts. The statistical correction may be determined by means of calibration of a measurement apparatus. As an alternative, the statistical correction may be determined at a general level for setting up measurement apparatuses to be used in connection to the sample acquiring device **10**. This statistical correction is of similar magnitude as statistical corrections that are presently performed in analysis apparatus that use the Coulter principle. It is contemplated that the sample acquiring device **10** could be used to analyse white blood cell counts as large as 50×10^9 cells/litre blood.

A surface of a wall of the measurement cavity **20** is at least partly coated with a reagent **22**. The reagent **22** may be freeze-dried, heat-dried or vacuum-dried and applied to the surface of the measurement cavity **20**. When a blood sample is acquired into the measurement cavity **20**, the blood will make contact with the dried reagent **22** and initiate a reaction between the reagent **22** and the blood.

The reagent **22** is applied by inserting the reagent **22** into the measurement cavity **20** using a pipette or dispenser. The reagent **22** is solved in a volatile liquid, e.g. an organic solvent such as methanol, when inserted into the measurement cavity **20**. The solvent with the reagent **22** may fill the measurement cavity **20**. Then, drying is performed such that the solvent will be evaporated and the reagent **22** will be attached to the surfaces of the measurement cavity **20**.

Since the reagent is to be dried onto a surface of a narrow space, the liquid will have a very small surface in contact with ambient atmosphere, whereby evaporation of the liquid is rendered more difficult. Thus, it is advantageous to use a volatile liquid, such as methanol, which enables the liquid to be evaporated in an effective manner from the narrow space of the measurement cavity.

According to an alternative manufacturing method, the sample acquiring device **10** may be formed by attaching two pieces to each other, whereby one piece forms the bottom wall of the measurement cavity **20** and the other piece forms the top wall of the measurement cavity **20**. This allows a reagent **22** to be dried onto an open surface before the two pieces are attached to each other. Thus, the reagent **22** may be solved in water, since the solvent need not be volatile.

The reagent **22** comprises a hemolysing agent and a staining agent. The hemolysing agent may be a quaternary ammonium salt, a saponin, a bile acid, such as deoxycholic acid, a digitoxin, a snake venom, a glucopyranoside or a non-ionic detergent of type Triton. The staining agent may be Hematoxylin, Methylene blue, Methylene green, Methylene azure, cresyl violet acetate, Toluidine blue, Gentian violet, a Sudan analogue, Gallocyanine, or a Fuchsin analogue, or any combination thereof. When a blood sample makes contact with the reagent **22**, the hemolysing agent will act to lyse the red blood cells such that the lysed red blood cells are mixed with the blood plasma. Further, the staining agent will accumulate in the nuclei of the white blood cells. The reagent **22** should contain sufficient amounts of staining agent to distinctly stain all the nuclei of the white blood cells. Thus, there will often be a surplus of staining agent, which will be intermixed in the blood plasma. The surplus of staining agent will give a homogenous, low background level of staining agent in the blood plasma. The accumulated staining agent in the white blood cells will be distinguishable over the background level of staining agent.

The reagent **22** may also comprise other constituents, which may be active, i.e. taking part in the chemical reaction with the blood sample, or non-active, i.e. not taking part in the chemical reaction with the blood sample. The active constituents may e.g. be arranged to catalyse the hemolysing or staining action. The non-active constituents may e.g. be arranged to improve attachment of the reagent **22** to the surface of a wall of the measurement cavity **20**.

Within a few minutes, the blood sample will have reacted with the reagent **22**, such that the red blood cells have been lysed and the staining agent has accumulated in the nuclei of the white blood cells.

Referring to FIG. **2**, another embodiment of the sample acquiring device will be described. The sample acquiring device **110** comprises a chamber **120** forming the measurement cavity. The sample acquiring device **110** has an inlet **118** into the chamber **120** for transporting blood into the chamber **120**. The chamber **120** is connected to a pump (not shown) via a suction tube **121**. The pump may apply a suction force in the chamber **120** via the suction tube **121** such that blood may be sucked into the chamber **120** through the inlet **118**. The sample acquiring device **110** may be disconnected from the pump before measurement is performed. Like the measurement cavity **20** of the sample acquiring device **10** according to the first embodiment, the chamber **120** has a well-defined thickness defining the thickness of the sample to be examined. Further, a reagent **122** is applied to walls of the chamber **120** for reacting with the blood sample.

Referring now to FIG. **3**, an apparatus **30** for volumetric enumeration of white blood cells will be described. The apparatus **30** comprises a sample holder **32** for receiving a sample acquiring device **10** with a blood sample. The sample holder **32** is arranged to receive the sample acquiring device **10** such that the measurement cavity **20** of the sample acquiring device **10** is correctly positioned within the apparatus **30**. The apparatus **30** comprises a light source **34** for illuminating the blood sample within the sample acquiring device **10**. The light source **34** may be an incandescent lamp, which irradiates light in the entire visible spectrum. The staining agent which is accumulated in the nuclei of the white blood cells will absorb light of specific wavelengths, such that the nuclei of the white blood cells will emerge in a digital image of the sample. If a colour image is acquired, the white blood cells will emerge as specifically coloured dots. If a black and white image is acquired, the white blood cells will emerge as dark dots against a lighter background.

The light source **34** may alternatively be a laser or a light emitting diode. This may be used for increasing contrast in the image such that the white blood cells may be more easily detected. In this case, the light source **34** is arranged to radiate electromagnetic radiation of a wavelength that corresponds to an absorption peak of the staining agent. The wavelength should further be chosen such that the absorption of the blood compounds is relatively low. Further, the walls of the sample acquiring device **10** should be essentially transparent to the wavelength. For example, where Methylene blue is used as the staining agent, the light source **34** may be arranged to irradiate light having a wavelength of 667 nm.

The apparatus **30** further comprises an imaging system **36**, which is arranged on an opposite side of the sample holder **32** relative to the light source **34**. Thus, the imaging system **36** is arranged to receive radiation which has been transmitted through the blood sample. The imaging system **36** comprises a magnifying system **38** and an image acquiring means **40**. The magnifying system **38** is arranged to provide a magnifying power of 3-200×, more preferably 3-100×, and most preferably 3-4×. Within these ranges of magnifying power, it is possible to distinguish the white blood cells. The image may be acquired with an improved resolution in order to allow lower magnifying power to be used. Further, the depth of field of the magnifying system **38** may still be arranged to at least correspond to the thickness of the measurement cavity **20**.

The magnifying system **38** comprises an objective lens or lens system **42**, which is arranged close to the sample holder **32**, and an ocular lens or lens system **44**, which is arranged at a distance from the objective lens **42**. The objective lens **42** provides a first magnification of the sample, which is further magnified by the ocular lens **44**. The magnifying system **38** may comprise further lenses for accomplishing an appropriate magnification and imaging of the sample. The magnifying system **38** is arranged such that the sample in the measurement cavity **20** when placed in the sample holder **32** will be focussed onto an image plane of the image acquiring means **40**.

The image acquiring means **40** is arranged to acquire a digital image of the sample. The image acquiring means **40** may be any kind of digital camera, such as a CCD-camera. The pixel size of the digital camera sets a restriction on the imaging system **36** such that the circle of confusion in the image plane may not exceed the pixel size within the depth of field. However, the white blood cells may still be detected even if they are somewhat blurred and, therefore, the circle of confusion may be allowed to exceed the pixel size while being considered within the depth of field. The digital camera **40** will acquire a digital image of the sample in the measurement cavity **20**, wherein the entire sample thickness is sufficiently focussed in the digital image for counting the white blood cells. The imaging system **36** will define an area of the measurement cavity **20**, which will be imaged in the digital image. The area being imaged together with the thickness of the measurement cavity **20** defines the volume of the sample being imaged. The imaging system **36** is set up to fit imaging blood samples in sample acquiring devices **10**. There is no need to change the set-up of the imaging system **36**. Preferably, the imaging system **36** is arranged within a housing such that the set-up is not accidentally changed.

The apparatus **30** further comprises an image analyser **46**. The image analyser **46** is connected to the digital camera **40** for receiving digital images acquired by the digital camera **40**. The image analyser **46** is arranged to identify patterns in the digital image that correspond to a white blood cell for counting the number of white blood cells being present in the digital image. Thus, the image analyser **46** may be arranged to identify dark dots in a lighter background. The image analyser **46** may be arranged to first electronically magnify the digital image before analysing the digital image. This implies that the image analyser **46** may be able to more easily distinguish white blood cells that are imaged closely to each other, even though the electronic magnifying of the digital image will make the digital image somewhat blurred.

The image analyser **46** may calculate the number of white blood cells per volume of blood by dividing the number of white blood cells being identified in the digital image with the volume of the blood sample, which is well-defined as described above. The volumetric white blood cell count may be presented on a display of the apparatus **30**.

The image analyser **46** may be realised as a processing unit, which comprises codes for performing the image analysis.

Referring to FIG. **4**, a method for volumetric enumeration of white blood cells will be described. The method comprises acquiring a blood sample in a sample acquiring device, step **102**. An undiluted sample of whole blood is acquired in the sample acquiring device. The sample may be acquired from capillary blood or venous blood. A sample of capillary blood may be drawn into the measurement cavity directly from a pricked finger of a patient. The blood sample makes contact with a reagent in the sample acquiring device initiating a reaction. The red blood cells will be lysed and a staining agent is accumulated in the nuclei of the white blood cells. Within a few minutes from acquiring the blood sample, the sample is ready to be analysed. The sample acquiring device is placed in an analysis apparatus, step **104**. An analysis may be initiated by pushing a button of the analysis apparatus. Alternatively, the analysis is automatically initiated by the apparatus detecting the presence of the sample acquiring device.

The sample is irradiated, step **106**, and a digital image of a magnification of the sample is acquired, step **108**. The sample is being irradiated with electromagnetic radiation of a wavelength corresponding to an absorption peak of the staining agent. This implies that the digital image will contain black or darker dots in the positions of the white blood cell nuclei.

The acquired digital image is transferred to an image analyser, which performs image analysis, step **110**, in order to count the number of black dots in the digital image.

In FIG. **5**, an example of a digital image is shown to indicate the possibility to identify white blood cells in a blood sample which is hemolysed and stained. This digital image was obtained of a sample acquiring device having a cavity thickness of 140 μm and using 50 times magnification. The light source irradiates white light, indicating that the white blood cells may be identified even though the irradiation is not specifically adapted to an absorption peak of the staining agent. The staining agent used was Methylene blue. Distinct black dots appear in FIG. **5** indicating white blood cells. The image shown in FIG. **5** is a black and white version of a colour image. The contrast between the white blood cells and the background appears clearer in the colour image than in the black and white image reproduced here. The black dots may easily be counted by an image analyser.

In manual methods of counting white blood cells, approximately 200 cells are typically counted for determining the white blood cell count of the blood sample. The method and apparatus presented here may for example be arranged to count approximately 2000 cells, which gives better statistical certainty of the obtained results. A normal, healthy adult has a white blood cell count of $4-5\times10^9$ cells/litre blood. This implies that 2000 cells are found in samples having a volume of 0.4-0.5 μl. For example, if an area of 1.5×1.5 mm in the measurement cavity having a thickness of 140 μm is imaged, the volume being imaged is 0.315 μl. A part of the acquired image may be selected for analysis. Thus, the acquired image may first be coarsely analysed such that no anomalies are allowed in the part being used for determining the white blood cell count. The part of the acquired imaged selected for analysis may be selected having an appropriate size so that a sufficient volume of the blood sample will be analysed.

It should be emphasized that the preferred embodiments described herein is in no way limiting and that many alternative embodiments are possible within the scope of protection defined by the appended claims.

The invention claimed is:

1. A sample acquiring device for volumetric enumeration of white blood cells in a blood sample, said sample acquiring device comprising:

- a measurement cavity for receiving a blood sample, said measurement cavity having a predetermined fixed thickness, which is a uniform thickness of 50-170 micrometers,
- a reagent, which is arranged in a dried form on a surface defining the measurement cavity, said reagent comprising a hemolysing agent for lysing red blood cells in the blood sample, and a staining agent for selectively staining white blood cells in the blood sample.

2. The sample acquiring device according to claim **1**, wherein the sample acquiring device comprises a body member having two planar surfaces to define said measurement cavity.

3. The sample acquiring device according to claim **2**, wherein the planar surfaces are arranged at a predetermined distance from one another to determine a sample thickness for an optical measurement.

4. The sample acquiring device according to claim **1**, wherein the measurement cavity has a uniform thickness of at least 100 micrometers.

5. The sample acquiring device according to claim **4**, wherein the measurement cavity has a uniform thickness of no more than 150 micrometers.

6. The sample acquiring device according to claim **1**, wherein the measurement cavity has a uniform thickness of no more than 150 micrometers.

7. The sample acquiring device according to claim **1**, wherein the reagent has been applied to the surface solved in a volatile liquid which has evaporated to leave the reagent in a dried form.

8. The sample acquiring device according to claim **1**, wherein the staining agent is arranged to selectively stain the nucleus of the white blood cells.

9. The sample acquiring device according to claim **1**, wherein the staining agent is any one in the group of Hematoxylin, Methylene blue, Methylene green, Methylene azure, cresyl violet acetate, Toluidine blue, Gentian violet, Sudan analogues, Gallocyanine, and Fuschin analogues, or any combination thereof.

10. The sample acquiring device according to claim **1**, wherein the hemolysing agent is a quaternary ammonium salt, a saponin, a bile acid, a digitoxin, a snake venom, a glucopyranoside, or a non-ionic detergent of type Triton.

11. The sample acquiring device according to claim **1**, further comprising a sample inlet communicating the measurement cavity with the exterior of the sample acquiring device, said inlet being arranged to acquire a blood sample.

12. The sample acquiring device according to claim **1**, wherein the sample acquiring device is disposable.

13. A system for volumetric enumeration of white blood cells in a blood sample, said system comprising:

- a sample acquiring device having a measurement cavity according to claim **1**, and
- a measurement apparatus comprising:
  - a sample acquiring device holder arranged to receive the sample acquiring device which holds a blood sample in the measurement cavity,
  - a light source arranged to irradiate the blood sample,
  - an imaging system, comprising a magnifying system and a digital image acquiring means for acquiring a digital image of a magnification of the irradiated sample in the measurement cavity, wherein white blood cells are distinguished in the digital image by selective staining of the staining agent, and
  - an image analyser arranged to analyse the acquired digital image for identifying white blood cells and determining the number of white blood cells in the blood sample.

14. The system according to claim **13**, wherein the magnifying system is arranged with a depth of field of at least the thickness of the measurement cavity of the sample acquiring device.

15. The system according to claim **14**, wherein a volume of an analysed sample is well-defined by the thickness of the measurement cavity and an area of the sample being imaged.

16. The system according to claim **13**, wherein a volume of an analysed sample is well-defined by the thickness of the measurement cavity and an area of the sample being imaged.

17. The system according to claim **13**, wherein the light source is arranged to irradiate light of a wavelength corresponding to a peak in absorbance of the staining agent.

18. The system according to claim **13**, wherein said light source comprises a laser source.

19. The system according to claim **13**, wherein said light source comprises a light emitting diode.

20. The system according to claim **13**, wherein the magnifying system has a magnification power of 3-200×.

21. The system according to claim **13**, wherein the image analyser is arranged to identify areas of high light absorbance in the digital image.

22. The system according to claim **21**, wherein the image analyser is arranged to identify black dots in the digital image.

23. The system according to claim **13**, wherein the image analyser is arranged to electronically magnify the acquired digital image.

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